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Note

High-performance liquid chromatographic determination of *tert.*-butylhydroquinone in vegetable oils

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tert.-Butylhydroquinone (TBHQ) is used as an anti-oxidant in vegetable oils. It can be used alone or in combination with other synthetic anti-oxidants[1]. For the quantitative determination of TBHQ a spectrophotometric[2] or a gas chromatographic (GC) method[3] can be applied. Although the GC method is more specific it is time- and labour-consuming as it is necessary to extract and derivatize the TBHQ. Recently, a gel permeation chromatographic method for the analysis of TBHQ and other anti-oxidants was reported[4]. Analysis time for this method is *ca.* 30 min and the detection limit for TBHQ is 500 ng. We report here a rapid and specific high-performance liquid chromatographic (HPLC) method for the quantitative determination of TBHQ in oils.

EXPERIMENTAL

Reagents

Dioxane containing 0.1 g water/100 g, *n*-hexane (spectroscopic grade) containing 0.004 g water/100 g and Oxinex 2378 were obtained from Merck (Darmstadt, G.F.R.). TBHQ was obtained from Eastman (Kingsport, Tenn., U.S.A.), butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and 2,4,5-trihydroxybutyrophenone (TBP) from BDH (Poole, Great Britain) and dodecyl gallate (DG) from Naarden (Wormerveer, The Netherlands).

Equipment

The HPLC equipment consisted of a Varian 5000 pump, Valco inlet valve and a Farrand Mk 1 spectrofluorometer, equipped with a 7-μl flow cell. A primary wavelength of 309 nm, a secondary wavelength of 340 nm and a slit width of 10 nm were used. Peak retention times and areas were determined with a Hewlett-Packard 3352B laboratory data system. The stainless steel column (25 × 0.4 cm I.D.) was slurry-packed[5] with 5 μm LiChrosorb SI 60 (Merck).

Method

The mobile phase consisted of dioxane and *n*-hexane (24:76, v/v), pumped at a flow-rate of 3 ml/min. TBHQ standard solutions containing 1, 2, 2.8, 4 and 10

mg/100 ml in *n*-hexane were prepared. A calibration curve (peak areas against concentration) was obtained by injecting 13.9 μl of each standard solution in duplicate. TBHQ in oil samples was determined by direct injection of 13.9 μl oil. Recovery determinations were conducted by adding 20 mg TBHQ to 100 g maize oil (oil density 0.918 g/cm^3 at 20°).

RESULTS AND DISCUSSION

A typical chromatogram of TBHQ dissolved in oil is reproduced in Fig. 1. TBHQ eluted with a capacity factor of $k' = 2.1$ from a column giving 4600 theoretical plates. The maize oil tocopherols, which are also detectable under the prescribed conditions of analysis, eluted with a capacity factor close to zero. An oil sample without TBHQ did not show any peaks at or near $k' = 2.1$.

Solutions of Oxinex 2378, BHT, BHA, DG and TBP in *n*-hexane were prepared and injected. Only BHA gave a detectable peak which eluted with a k' value similar to that of the tocopherols. Subsequently a mobile phase of 4% dioxane in

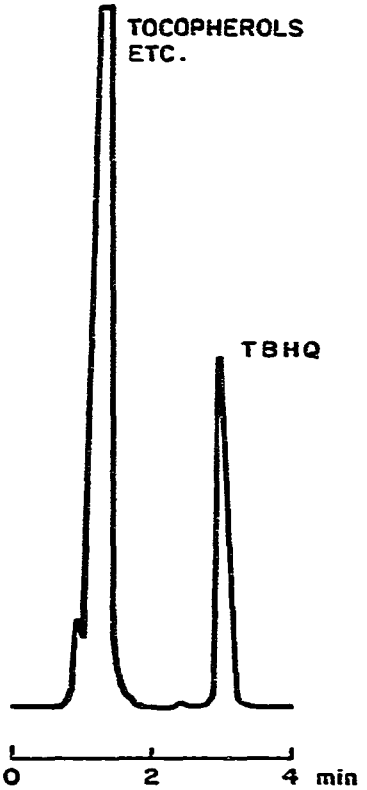

Fig. 1. Chromatogram of 20 mg TBHQ/100 g maize oil. Steel column (25 × 0.4 cm I.D.) slurry-packed with LiChrosorb SI 60 (5 μm), with dioxane and *n*-hexane (24:76) as mobile phase (3 ml/min) and a fluorometric detector.

n-hexane, which was more suitable for the separation of the tocopherols[6], was used and we found that BHA eluted with γ-tocotrienol.

A linear calibration curve for TBHQ was obtained and the least-squares method was applied to calculate the line of best fit for the data ($y = 48.3x - 2.2$, $r = 0.9998$). A recovery figure of 98.5% for TBHQ from maize oil was obtained with a coefficient of variation of 2.4% for eight determinations. The detection limit was 6 ng, and the average analysis time per sample was 5 min. The described method is therefore fast, sensitive and convenient.

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